

## SUPPLEMENTAL MATERIAL

LeBlanc et al., <https://doi.org/10.1084/jem.20161802>**A Human VEGF<sub>1-110</sub> coding sequence with altered codons**

(65.8% identical human VEGF and 66.4% to mouse VEGF)

Human VEGF	GCACCCATGGCAGAAGGAGGAGGGCAGAATCATCACGAAGTGGTGAAGTTCATGGATGTC	60
Altered codon	GCTCCGATGGCTGAGGGTGGTGGCCAAAACCACCATGAGGTTGTCAAATTTATGGACGTG	60
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Human VEGF	TATCAGCGCAGCTACTGCCATCCAATCGAGACCCTGGTGGACATCTTCCAGGAGTACCCT	120
Altered codon	TACCAACGTTCTTATTGTCCACCTATTGAACTTTAGTTGATATTTTCAAGAATATCCA	120
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Human VEGF	GATGAGATCGAGTACATCTTCAAGCCATCCTGTGTGCCCTGATGCGATGCGGGGGCTGC	180
Altered codon	GACGAAATGAATATATTTTAAACCTAGCTGCGTCCATTGATGCGTTGTGGTGGATGT	180
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Human VEGF	TGCAATGACGAGGGCCTGGAGTGTGTGCCACTGAGGAGTCCAACATCACCATGCAGATT	240
Altered codon	TGTAACGATGAAGGTTTGGAAATGCGTTCCAACAGAAGAAAGCAATATACGATGCAAATC	240
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Human VEGF	ATGCGGATCAAACCTCACCAAGGCCAGCACATAGGAGAGATGAGCTTCCTACAGCACAAC	300
Altered codon	ATGCGTATTAAGCCACATCAGGGTCAACATATGGTGAATGTCTTTTACAACATAAT	300
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Human VEGF	AAATGTGAATGCAGACCAAAGAAAGATAGA	330
Altered codon	AAGTGCAGTGTGTCCTAAAAAGGACCGT	330
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**B Amino acid sequence alignment**(100% identical to human VEGF<sub>1-110</sub>)

Wild-type	APMAEGGGQNHHEVVKFMDVYQRSYCHPIETLVDIFQEYPDEIEYIFKPSCVPLMRCGGC	60
Altered codon	APMAEGGGQNHHEVVKFMDVYQRSYCHPIETLVDIFQEYPDEIEYIFKPSCVPLMRCGGC	60
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Wild-type	CNDEGLECVPTESNITMQIMRIKPHQGQHIGEMSFLQHNKCECRPKKDR	110
Altered codon	CNDEGLECVPTESNITMQIMRIKPHQGQHIGEMSFLQHNKCECRPKKDR	110
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Figure S1. **VEGF-Phage displays wild-type human VEGF protein but with artificial codons.** (A) The coding sequence with altered codons is 65.8 and 66.4% identical to human and mouse VEGF. (B) The encoded protein is 100% identical to human VEGF<sub>1-110</sub> (NCBI RefSeq accession no. NM\_001171623). Altered codons were optimized for bacteria expression and phage display. Asterisks indicate identical nucleotides or amino acids.

Table S1. List of known or putative endothelial ligands identified by comparative ligandomics

CCDS ID	Protein	Binding activity		Activity ratio	Comments and references
		Control	DR		
CCDS23347	Scg3	0	1,731	1,732	Scg3 is verified as a DR-associated angiogenic factor in this study.
CCDS18810	C1qb	0	837	838	C1qb is the $\beta$ subunit of C1q, which is a known EC ligand (Kishore and Reid, 2000).
CCDS28285	App	1	206	103.5	APP-derived $\beta$ -amyloid binds to RAGE (see Results).
CCDS26200	Jag2	0	92	93	Jag2 is a ligand for Notch1, which is expressed on ECs (Kume, 2012).
CCDS20005	Ptn	0	38	39	Ptn is a known angiogenic factor involved in the pathogenesis of PDR (Zhu et al., 2015).
CCDS15089	Scg2	0	16	17	Scg2 is a prohormone of secretoneurin, which is an angiogenic factor (Kirchmair et al., 2004).
CCDS15806	Notch1	0	16	17	Notch1 is a ligand for Jagged1, which is expressed on ECs (Kume, 2012).
CCDS19483	Sparcl1	1,003	5,336	5.3157	Sparc modulates cell cycle progress in bovine aortic ECs (Funk and Sage, 1991).
CCDS19983	Podxl	3,877	9,349	2.4110	Podxl2 is an L-selectin ligand that is expressed on ECs (Fieger et al., 2003).
CCDS16777	Chgb	3,531	3,910	1.1073	CgB, a member of the granin family, has an undefined role in EC regulation.
CCDS51013	Notch2	2,114	2,264	1.0709	Notch2 is a ligand for Jagged1, which is expressed on ECs (Kume, 2012).
CCDS28578	Tulp1	55,252	49,334	0.8929	Tulp1 is a ligand for TAM receptor tyrosine kinase family, similar to Gas6 (see Gas6 below; Caberoy et al., 2010; Lemke, 2013)
CCDS40232	Gas6	8,876	7,139	0.8043	Gas6 is a ligand for the TAM family of Tyro3, Axl, and Mer receptor tyrosine kinases to regulate ECs (Lemke, 2013).
CCDS20912	Apoe	90	61	0.6813	Apolipoprotein E is a potent inhibitor of EC proliferation (Vogel et al., 1994).
CCDS28390	Plg	35	11	0.3333	Plasminogen binds to EC surface actin to regulate fibrinolysis (Li et al., 1996).
CCDS16766	Prnp	220	47	0.2172	Prion protein promotes angiogenesis (Turu et al., 2008).
CCDS24864	Ntn1	11	0	0.0833	Netrin-1 inhibits EC apoptosis (Castets et al., 2009).
CCDS17149	Gnas	71	0	0.0139	Gnas inhibits endothelial proliferation (Sakamoto et al., 2009).
CCDS18100	Tpm2	12,226	165	0.0136	Tpm2 is highly homologous to tropomyosin, which extrinsically inhibits angiogenesis (Doñate et al., 2004).
CCDS17457	Hdgf	83	0	0.0119	HDGF is an angiogenic factor (Oliver and Al-Awqati, 1998; LeBlanc et al., 2016).
CCDS40011	Hdgfrp3	11,140	48	0.0044	Hdgfrp3 or HRP-3 is an angiogenic factor (LeBlanc et al., 2015).
CCDS16498	Rcn1	23,107	71	0.0031	Rcn1 binds to EC surface (Cooper et al., 2008).
CCDS14820	Sulf1	338	0	0.0030	Sulfatase 1 remodels 6-O-sulfation on cell surface and inhibits VEGF signaling and angiogenesis (Lai et al., 2008).

Inclusion criteria: binding activity >10 (i.e., the activity of GFP-Phage) in diabetic or control retina.

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